the Sequence Listing. Applicants respectfully request examination on the merits of this application.

Respectfully submitted,

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January 14, 2002
Date

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Marked-Up version of specification paragraphs:

Page 8, last full paragraph:

The present invention further is directed to a regulatable element to which AAV Rep78 binds and controls, and a regulatable promoter containing this sequence that preferably includes nucleotides 14-56 as shown in Figure 2 **(SEQ ID NO:4).**

Page 9, second full paragraph:

Figure 2 shows the sequences of the HPV-16 p97 promoter **(SEQ ID NO:4)** with the important elements within the immediate p97 region as labeled boxes.

Page 11, sixth full paragraph:

Figure 15A-C discloses the nucleotide sequence of the AAV2 genome **(SEQ ID NO:5)** as disclosed in Srivastava, A. *et al., J.Virol.* 45:555 (1983). The nucleotide sequence encoding AAV Rep78 is nucleotides 321-2186, in which nucleotides 2184-2186 are the stop codon, taa.

Page 11, seventh full paragraph:

Figure 16 discloses the 621 amino acid sequence of AAV Rep78 **(SEQ ID NO:6)** encoded by nucleotides 321-2183 as discussed above in the description of Figure 15.

Page 14, first full paragraph:

The present invention provides sufficient guidance to a person skilled in the art to obtain additional AAV Rep78 mutants that have modified binding affinities as compared to the wild-type AAV Rep78. The nucleic acid sequence is disclosed in Srivastava *et al.*.*J.Virol.* 45:555 (1983) and is disclosed in Figure 15A-C **(SEQ ID NO:5)** which is used to prepare mutants according to the present invention. AAV has been studied and the organization of the AAV genome is disclosed in Muzyczka, N. *et al.*, *Current Topics in Microbiology and Immunology* 158: 97-129 (1992), particularly Figure 3. The present invention discloses a sequence to which the AAV Rep78 binds to in Figure 2 **(SEQ ID NO:4)** and discloses methods to test for the strength of the binding or the lack of binding to

DNA sequences, preferably promoter regions. The present invention is intended to encompass other AAV Rep78 mutants that have modified DNA binding; for example, promoter binding affinities. Such methods would not require undue experimentation and are disclosed in the present invention or known to skilled persons.

Page 18, first full paragraph:

The present invention also discloses an AAV Rep78 regulation element comprising the nucleotides shown in the nucleotide sequence of Figure 2 **(SEQ ID NO:4)**, wherein this element provides the binding site for the AAV Rep78 protein, and particularly comprises about nucleotides 14 –56 of the nucleotide sequence of Figure 2 **(SEQ ID NO:4)**. This present invention also discloses an AAV Rep78 regulatable promoter comprising the regulation element of the nucleotides shown in the nucleotide sequence of Figure 2 **(SEQ ID NO:4)**, wherein this element provides the binding site for the AAV Rep78 protein, and particularly comprises about nucleotides 14 –56 of the nucleotide sequence of Figure 2 **(SEQ ID NO:4)**. This AAV Rep78 regulatable promoter comprises the regulation element described above and the remaining promoter sequences from a promoter other than the HPV-16 p97 promoter.

Page 19, second full paragraph that bridges to page 20:

Construction of AAV Rep78 mutant plasmids and production of MBP-AAV Rep78 chimeric proteins

The construction of any of the AAV Rep78 mutants utilizes the know AAV Rep78 nucleic acid sequence as disclosed, for example, in Figure 15A-C **(SEQ ID NO:5)** and in Srivastava *et al.*, *J. Virol.* 45:555 (1983). Further, the 621 amino acid wild-type Rep78 encoded by nucleotides 321-2183 (stop codon nucleotides 2184-2186) is disclosed in Figure 16 (SEQ ID NO:6) and was known. The construction of the plasmid pMAL-Rep-$64^{LH}65^{TM}$ from which mutant MBP-$64^{LH}65^{TM}$ protein is produced, has been described previously (Batchu, *et al.*, *Biochem. Biophy. Res. Comm.* 208:714-720 (1995), Batchu, *et al.*, *Biochem. Biophy. Res. Comm.* 210:717-725 (1995)). The plasmid pMal-Rep-77^{LG} was similarly constructed using a different mutagenic oligonucleotide **(SEQ ID NO:1)** (5'-CCCCGGAGGCCGAATTCTTTGTGCAA) and the M13 based plasmid pALTER-AAV3 (containing all of the AAV genes). A second oligonucleotide created a *Sph* I restriction site

immediate upstream of AAV Rep78 ORF. The mutations were initially characterized by the generation of a new restriction site and were further verified by DNA sequencing with Sequenase (U.S. Biochemicals) according to the manufacturer's recommendations. The mutant AAV Rep78 ORFs were then transferred into pMALc2 on an *Sph* I and *Xho* I fragment (nt. 321 to nt. 2233) to generate pMAL-Rep-$64^{LH}65^{TM}$ and pMAL-Rep-77^{LG}. Both the mutant and wild-type fusion proteins with MBP were purified by affinity chromatography using amylose resin following kit directions (Protein Purification and Expression System, New England Biolabs). Fractions were collected and analyzed by SDS-polyacrylamide gels. Purified fractions were concentrated using Centricon 10 kDa cut-off membrane filters (Amicon). Routinely these procedures resulted in MBP-AAV Rep78 and $64^{LH}65^{TM}$ proteins of 70-90% purity with a yield of 20 µg/100ml bacterial culture.

Page 20, second full paragraph:

In vitro transcription analysis of p97 promoter activity

An HPV-16 p97-CAT DNA fragment was used as a template for transcription. The p97-CAT DNA fragment was generated by PCR amplification using primer 1 **(SEQ ID NO:2)** (5'ACAAGCAGGATTGAAGGCCA, HPV-16 nt 7043-7065) complementary to the p97 sequences) and primer 2 **(SEQ ID NO:3)** (5' CATATCACCAGC TCACCGTC, nt 615-633 of pSV2CAT) complementary to the CAT sequences). The plasmid p16P (p97-CAT) was used as the original PCR template (Romanczuk, *et al.*, *J. Virol.* 64:2849-2859 (1990)). This produced a 1.2 Kb product. A 25 µl reaction mixture contained 0.5 µg of DNA template, 20 mM HEPES pH 7.9, 5 mM $MgCl_2$, 100 mM KCl. 0.5 mM DTT, 20% glycerol, 25 µM [^{32}P] GTP, 400 µM ATP, CTP and UTP, and 8 units Hela nuclear extract (Promega, HPV positive cervical cancer) or 5 µg of T-47D nuclear extract (Geneka Corp., HPV negative breast ductal carcinoma). Reactions were incubated at 30^0C for 60 min, and then terminated by adding 175 µL of Stop Solution containing 300 mM Tris-HCl, pH 7.9, 0.5% SDS, 300 mM sodium acetate, 2 mM EDTA and 3 µg/ml tRNA. RNA was extracted with phenol-chloroform, precipitated with ethanol, and finally dissolved in 10 µL of formamide containing 0.1% each of xylene cyanol and bromophenol blue. Samples were analyzed on an 6% polyacrylamide, 7 M urea gel, died, analyzed by autoradiography. An p97-specifc RNA product of approximately 300 bases results.

<u>Page 21, the fifth full paragraph bridging over to page 22:</u>

Within the 1.8 kb fragment lies the long control region (LCR) of HPV-16 which contains central *cis* elements (origin of replication [*ori*], enhancers and promoters) essential for HPV-16 biological function. Furthermore, AAV Rep78 is a viral transcription factor known to bind promoter DNA (McCarty, *et al.*, *J. Virol.* 74:4988-4997 (1994), Batchu, *et al.*, *Cancer Letters* 86:23-31 (1994), Wonderling, *et al.*, *J. Virol.* 71:2528-2534 (1996)). Thus, it is reasoned that AAV Rep78 might be targeting the *ori*/p97 region within this fragment due to AAV Rep78's known modulation of the HPV-16 p97 and HPV-18 p105 promoters (Hermonat, P.L., *Virology* 172:253-261 (1989), Hermonat, P.L., *Cancer Research* 54:2278-2281 (1994), Hermonat, *et al.*, *Gynecologic Oncology* 66:487-494 (1997), Horer, *et al.*, *J. Virol.* 69:5485-5496 (1995)), and of BPV-1 DNA replication (Hermonat, P.L. *Virology* 189:329-333 (1992)). To map the region of binding sequentially smaller substrates from this region were tested for recognition by AAV Rep78 (Figures 1B-C) by EMSA analysis. In Figure 1B AAV Rep78 was shown to strongly recognize the HPV-16 sequences from nt 7814-106 (p97), while it does not significantly recognize a similarly sized analogous fragment from the MSV-LTR. These data clearly indicate that there is a specific recognition of the p97 DNA well above non-specific binding. As mentioned earlier, AAV Rep78 is able to inhibit expression from p97, but has little effect on the MSV-LTR (Hermonat, P.L., *Cancer Research* 51:3373-3377 (1991)). Thus, AAV Rep78 binding of promoter DNA may be associated with an ability to regulate p97 expression. In Figure 1C, the target sequence was further defined to be in the 3' half of this region (nt 14-106, hereafter referred to as "p97"). Finally, in Figure 1D, a strong target sequence for AAV Rep78 binding is shown to be contained within nt 14-56. Figure 2 **<u>(SEQ ID NO:4)</u>** shows the sequences of this region. Note that the nt 14-56 sequences contain an intact E2 binding motif and an Sp1 binding motif. These sequences also partially overlap the HPV-16 *ori* and E1 binding regions.